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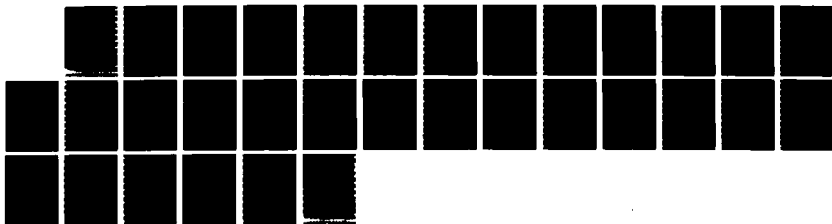
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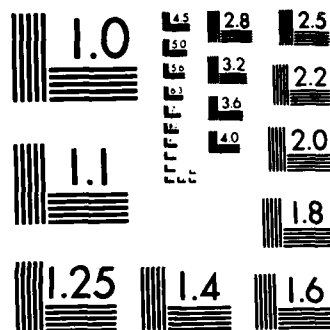
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**PRINCIPLES AND PROCEDURES  
FOR THE EVALUATION OF CHEMICAL AND  
BIOCHEMICAL SENSORS DURING  
EXPLORATORY DEVELOPMENT**

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DETECTION DIRECTORATE**

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## PREFACE

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# PRINCIPLES AND PROCEDURES FOR THE EVALUATION OF CHEMICAL AND BIOCHEMICAL SENSORS DURING EXPLORATORY DEVELOPMENT

## 1. INTRODUCTION

This report describes fundamental principles and procedures to follow in the evaluation of chemical and biochemical sensors during exploratory development (military development at the 6.2 level). The principles and procedures deal most closely with sensors that detect chemical, immunological, or physical interactions on a selective surface using optical, electrochemical, piezoelectric, or some other transduction principle.

The term "sensor" as used hereafter refers to this selective surface and to the other physical components directly involved in the transduction of chemical information into an electrical signal. Brief mention will be made of other aspects of a measurement system such as reference elements, electronic circuitry, and sample-handling devices; these components together with the sensor will hereafter be referred to as a "sensor system." The system's amenability to new applications will also be discussed. The procedures are to enable users to determine how well the sensor works in a laboratory setting and to establish the sensor's sensitivity to conditions and interferences that may be encountered in actual military use. When these procedures are applied to a sensor obtained commercially or from a contractor, the procedures should enable users to verify that the sensor behaves as the developer states and that the sensor design is reasonably optimized according to the criteria presented in this report. In addition, the tests could determine whether the sensor system can be used for military applications other than those for which it was originally designed.

The tests discussed in the text do not have to be conducted in the sequence in which they are presented. However, the order is appropriate unless one particular system component or environmental factor is obviously troublesome, in which case that component or factor should be investigated early on. If a previously unknown sensitivity is found during a particular test, previous tests may need to be repeated while controlling the sensitive parameter.

## 2. RESPONSE TO ANALYTE

By practical definition, a useful sensor must respond to the presence of a substance to be monitored, and this response must be distinguishable from the sensor's response to other expected changes in the sensor's environment. This section deals with the first of these criteria, while Sections 3 through 6 deal with the latter question.

Sensor response to the primary analyte (the agent that is to be detected) should be measured while keeping other reaction conditions (temperature, pH, ionic strength, buffer capacity,

flow rate, etc.) as constant as possible. Several different experimental methods are available to do this. In "standard addition," the sensor is immersed in a beaker of buffer, and aliquots of concentrated analyte are added to the beaker in order to expose the sensor to increasingly high concentrations of the analyte. Sensor output is monitored and compared against the calculated analyte concentrations. Alternatively, the sensor can be immersed in preformulated samples of differing analyte concentrations. It is best to start with the lowest concentration, and work towards the highest concentration; equilibration time will be minimized if this order is followed. A third method is to rinse or wash the sensor in between exposing it to different concentrations of the analyte. Section 8.3 gives information regarding the rinsing procedure.

To evaluate sensors designed for one-time use (e.g., some types of competitive immunoassays), first determine the homogeneity of a batch of sensors by testing several sensors with the same concentration of analyte. After determining the precision that can be expected from the method, test several different concentrations of analyte using a new sensor for each trial.

## 2.1 Choice of Analyte Concentrations.

For many physical and chemical sensing processes, the sensor output will be linearly dependent on the logarithm of analyte activity over a certain range of activity. In these cases and in the case of sensors having very large dynamic ranges, it is desirable to evaluate analyte concentrations at decade intervals (0.01 ppm, 0.1 ppm, 1 ppm, 10 ppm, and 100 ppm, for example). The selected concentrations should determine the dynamic range of the sensor. Once the dynamic range of the sensor is determined, additional intermediate concentrations within that dynamic range can be tested. Depending on the exact behavior of the sensor, usually triplicate analyses of 5 to 10 different concentrations are necessary for constructing a good calibration curve.

## 2.2 Determining the Lower Limit of Detection.

Factors determining the lower limit of detection include detector and amplifier noise, variations in sample presentation, constraints due to variations in biomolecular activities with time, and various random competitive physical processes on the sensor surfaces. In order to quantitatively compare different sensor sensitivities, it is advantageous to adopt a standard definition of limit of detection and a standard method for determining this limit.

The International Union of Pure and Applied Chemistry (IUPAC) defines the limit of detection as the concentration ( $C_L$ ) or amount ( $Q_L$ ) "calculated from the smallest measurement ( $X_L$ ) that can be detected with reasonable certainty for a given analytical procedure."<sup>1</sup> In other words, the limit of detection is

the smallest concentration or amount that can reasonably be distinguished from background. The IUPAC suggests the following method of calculating  $X_L$ :

$$X_L = X_{B,mean} + t s_B \quad (1)$$

where  $X_{B,mean}$  is the mean value of the blank responses, and  $s_B$  is the standard deviation (N-1 weighting) of the blank readings. The constant,  $t$ , is the value from Student's t-distribution corresponding to the confidence level desired and the number of degrees of freedom granted by the data.

The corresponding concentration is calculated from:

$$C_L = (X_L - X_{B,mean})/m \quad (2)$$

or

$$C_L = t s_B / m \quad (3)$$

where  $m$  is the analytical sensitivity (slope of  $x$  versus  $c$ ). This value will hereafter be referred to as the "detection limit." It corresponds to the smallest signal level that will not yield a significant fraction of "false positives" and is referred to as the "critical level" in some of the literature.<sup>2,3</sup>

Equations (1), (2), and (3) do not take into consideration any of the following effects: nonlinearity between the output variable and the concentration, error present in the determination of the slope and intercept of this relationship, and differences in the variances of measurements at different concentrations.<sup>1,2</sup> Nevertheless, the calculation is simple; and for comparison purposes, it is recommended that  $C_L$  at the 99% confidence level be reported in the evaluation of each sensor system, supplemented by other information as described below.

When there is significant uncertainty in the slope and intercept of the empirical relationship between the signal and concentration, a calculation of the limit of detection should be made taking these other sources of error into consideration.<sup>1</sup> The following expression can be used to calculate the value of  $C_L$ , using calculated values for the variances of the slope, intercept, and blank determinations ( $s_m$ ,  $s_i$ , and  $s_B$ , respectively):

$$C_L = (t/m) [s_B^2 + s_i^2 + ((i - x_{B,mean})/m)^2 s_m^2]^{1/2} \quad (4)$$

The parameters used in Equation (4) are defined in Table 1.

Table 1. Identification and Definition of Terms Used in the Calculation of the Detection Limit

---

All sums are for  $j = 1$  to  $n$ , unless otherwise noted.

| <u>Quantity</u>  | <u>Symbol</u>       | <u>Definition</u>  |
|--|---------------------|--|
| independent variable                                   | $C$                 |  |
| dependent variable                                     | $X$                 |  |
| individual measurements                                | $(C_j, X_j)$        |  |
| number of measurements, excluding blank determinations | $n$                 |  |
| blank determinations                                   | $X_{B,j}$           |  |
| number of blank determinations                         | $n_B$               |  |
| mean value of $X$                                      | $X_{\text{mean}}$   | $(\sum X_j)/n$   |
| mean value of $C$                                      | $C_{\text{mean}}$   | $(\sum C_j)/n$   |
| mean value of $X_B$                                    | $X_{B,\text{mean}}$ | $(\sum X_{B,j})/n$<br>for $j = 1$ to $n_B$                                     |
| slope of best-fit line                                 | $m$                 | $(n \sum C_j X_j) - ((\sum C_j)(\sum X_j))$<br>$(n \sum C_j^2) - (\sum C_j)^2$ |
| x-intercept of best fit line                           | $i$                 | $((\sum X_j)/n) - m((\sum C_j)/n)$   |
| sample variance  | $s^2$               | $(S_{XX} - mS_{CX})/(n-2)$   |
| variance of $X_{\text{mean}}$                          | $s^2_{\text{mean}}$ | $s^2/n$  |
| variance of slope                                      | $s^2_m$             | $s^2/S_{CC}$   |
| variance of intercept                                  | $s^2_i$             | $(s^2(\sum C_j^2))/(nS_{CC})$  |
| $\sqrt{\text{variance in } C}$                         | $S_{CC}$            | $(\sum C_j^2) - ((\sum C_j)^2/n)$  |
| $\sqrt{\text{variance in } X}$                         | $S_{XX}$            | $(\sum X_j^2) - ((\sum X_j)^2/n)$  |
| $\sqrt{\text{covariance}}$                             | $S_{CX}$            | $(\sum C_j X_j) - ((\sum C_j)(\sum X_j))/n$                                    |
| correlation coefficient                                | $r$                 | $S_{CX}/(\sqrt{S_{XX}S_{CC}})$   |

---

If the variances of measurements made at higher concentrations are larger than those made at lower concentrations, the measurements at the lower concentrations can be used in Equation (4), provided there are enough; or a method of weighting the variances can be employed.<sup>2</sup>

In cases where concentration is not a first-order, linear function of the sensor output, the slope ( $m$ ) in Equations (2), (3), and (4) has no meaning. However, Equation (1) can be used to calculate  $X_L$ ; the limit of detection ( $C_L$ ) can then be calculated from  $X_L$  using the equation which best describes the relationship between  $C$  and  $X$  (such as that obtained by nonlinear regression analysis).<sup>3</sup>

Figure 1 illustrates experimental data (designated by circles) for which the variance in the slope and intercept of the best-fit line has been calculated and graphed.<sup>4</sup> Any data collected will have a 99% probability of mapping into the area between the two dashed hyperbolae on the graph. The graph is constructed by first plotting the linear least squares line and marking the centroid of the data ( $C_{\text{mean}}, X_{\text{mean}}$ ). The variance in the slope and the mean ( $s_m$  and  $s_{\text{mean}}$ , respectively) are calculated using the formulae in Table 1. Straight lines through ( $C_{\text{mean}}, X_{\text{mean}}$ ) having slopes of  $m \pm t s_m$  are drawn, forming an "X." The two points ( $C_{\text{mean}}, X_{\text{mean}} \pm t s_{\text{mean}}$ ) (designated by triangles) are then located. A line is drawn through each of these points, asymptotically approaching the arms of the "X."  $X_L$  and  $C_L$  are also labeled for this set of data.

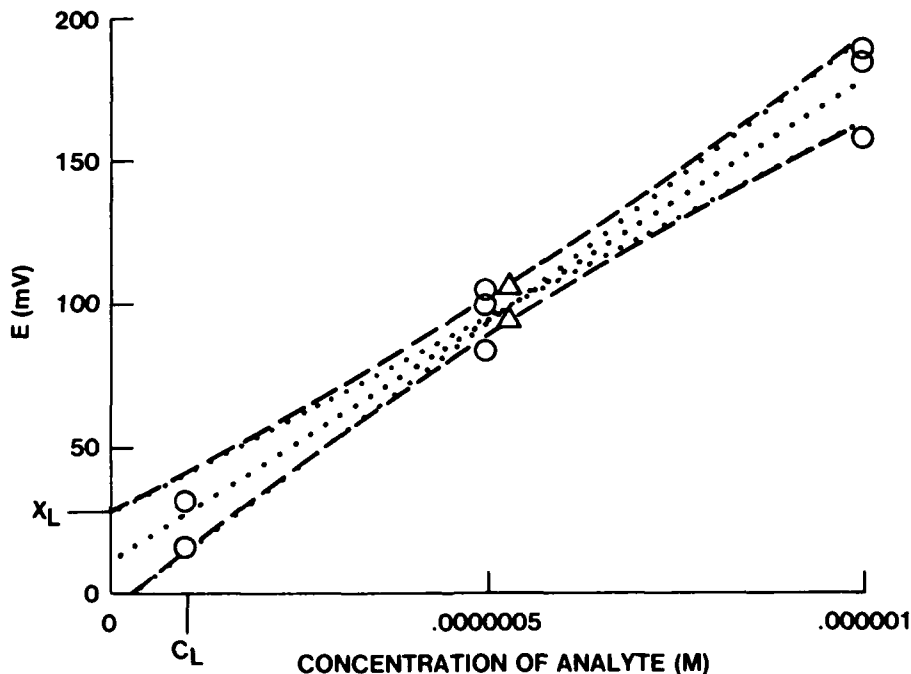


Figure 1. Calculated Variance in the Slope and Intercept of the Best-Fit Line for a Data Set

It should be noted that statistical calculations should not displace good experimental design. For example, analyses of concentrations near the proposed limit of detection need to be made to minimize the uncertainty in the response slope and variance in that area. The above formulae are not meant to be predictive, only to eliminate or reduce the subjectivity involved in the interpretation of actual data collected at or near the limit of detection.

### 3. SPECIAL EFFECTS TO MONITOR

Doing one series of tests, such as those described in Section 2, will not determine the baseline drift (rate at which sensor output for a specific analyte concentration is changing with respect to time) or the reversibility of the sensor response. Nor may other sources of anomalous shifts in sensor output be observed in such limited testing.<sup>5,6</sup> This section describes some effects to watch for during long-term continuous testing.

Baseline drift is measured by simply leaving the sensor in a sample of analyte at a concentration near the low end of the dynamic range of the sensor (a concentration where sensor operation is reliable) for an extended period of time. Temperature and other environmental factors must be closely controlled. If there is any possibility of the analyte adsorbing to the reaction vessel walls or being involved in other processes competitive with the sensor reaction mechanism, it is advisable to periodically or continuously replace the test solution with fresh solution of equal formulation. Sensor output over time is compared to the formulated concentration of the test solution. Analyte concentration may also be periodically verified by an independent analytical method.

Sudden shifts in sensor output might be seen during long-term drift studies. Sudden shifts in sensor output might also occur when the sensor is handled or when solution is rapidly passed over the sensor surface. Table 2 lists some possible causes of these offsets and suggestions for minimizing the offsets.

The hysteresis or irreversibility seen in the function of chemical sensors often has a component of true thermodynamic irreversibility (as does a liquid junction in an electrochemical cell)<sup>8</sup> and a component that is really a kinetic sensitivity: the response to an increase in analyte concentration may be much faster than the response to a decrease in analyte concentration. These effects are most often due to slow diffusion within a sensor coating or to a chemical reaction occurring which has a very high reverse activation energy. Figure 2 illustrates what may happen when a sensor is exposed to successively increasing analyte concentrations and then is exposed to successively decreasing analyte concentrations, allowing the same amount of time to elapse between a step change in concentration and the reading of the sensor output for each data point. The apparent



hysteresis may be due to the sensor taking longer to reach equilibrium when going from a higher to a lower concentration than when going from a lower to higher concentration. If this is the case, allowing more time to elapse between effecting the change in concentration and reading the sensor output will result in a smaller amount of hysteresis. Unfortunately, one does not want to wait for an extended period of time to make measurements, and often the uncertainty in the rate of drift and other effects in the sensor output make it impractical to wait. In a microprocessor-interfaced system, reproducible hysteresis effects can be compensated by calibration procedures; otherwise, these effects just add to the uncertainty in the interpretation of the sensor output.

Table 2. Possible Causes (and Remedies) for Sensor Offsets During Operation

| Cause  | Remedy  |
|--|---|
| Electrical charge buildup on sensor surface                            | Ground testing solution and testing personnel; isolate sensor system from ground using optical or inductive isolation amplifiers. |
| Electrostatic coupling to moving wiring or plastic tubing <sup>7</sup> | Avoid such movement; shield electronic components.  |
| Power surges   | Use surge protectors, regulated power supplies, or batteries.   |
| Gas bubbles forming in tubing  | Degas liquid reagents; avoid increases in temperature.  |
| Mechanical jostling  | Cushion vibration-sensitive components.   |

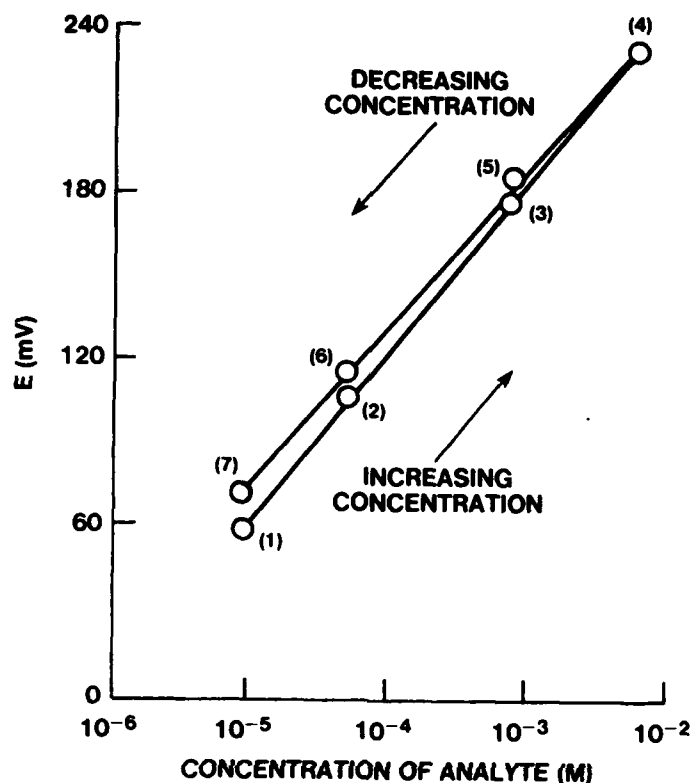


Figure 2. Hysteresis Effect. Data points are labeled in order in which they were collected.

#### 4. INTERFERING SUBSTANCES

Interfering substances are those that could bind the analyte; those that could competitively react with other sensor-related reagents; and those that could coat or inactivate electrodes, membranes, or other sensor surfaces. Some of the possible sources of interference are listed in Table 3 and include substances closely related to the primary analyte for the sensor, specific substances that can be expected to be present in a battlefield situation, and substances inherent to the sensor system itself (such as tubing) to which the sample is exposed. Interference may be manifest by shifts or increased drift in sensor output when the sensor is exposed to the potential interferent (at a constant concentration of the primary analyte). Interference may also be manifest as reduced response to subsequent changes in concentration of the primary analyte. Table 4 lists design considerations to reduce the effect of possible interferents.

Table 3. Possible Interferents to Which a Chemical or Biological Sensor Might be Exposed During Military Use

- 
1. Closely related proteins/genetic material (for immunological sensors)
  2. Chemicals with redox potentials near that of the analyte (for redox sensors)
  3. Strong oxidizing or reducing agents
  4. Strong acids or bases
  5. Heavy metals
  6. Complexing agents
  7. Detergents
  8. Tubing and other surfaces to which sample is exposed (due to possible adsorption of the analyte onto the surface)
  9. Smoke (tactical and from burning fuel, rubber, or vegetation)
  10. Diesel and gasoline fumes
  11. Dust
  12. Pollen
  13. Decontaminants
  14. Oils, insect repellent, and solvents incident to routine activities in a battlefield environment
-

Table 4. Design Considerations to Reduce Interference

- 
1. Filtering of sample to remove particulate matter.
  2. Sample preparation steps (such as liquid-liquid extraction) to separate the primary analyte from a matrix of possible interferents.
  3. Periodic washing, rinsing, and recalibration of sensor.
  4. Using chemically inert tubing and fittings, such as those made of fluoro-polymers.
  5. Pretreating tubing, etc., with silanes or other substances to alter the wetting properties of these surfaces.
  6. Pretreating tubing, etc., with albumin or other protein to reduce the binding of proteinaceous analytes to such surfaces.
  7. Coating of sensing surface in immunological sensors with neutral proteins (after loading with the immunoprotein of interest) in order to occupy unused binding sites and to reduce subsequent nonspecific binding.
- 

## 5. REACTION CONDITIONS

The sensor baseline reading and the magnitude of sensor response to a given change in analyte concentration are sometimes dependent on reaction conditions such as pH, ionic strength, temperature, and rate of mixing or of flow. These same parameters are also instrumental in determining the rate at which the sensor responds. It is desirable to determine the set of reaction conditions that will give optimum sensor performance with respect to stability, magnitude of response, speed of response, and practicality for intended use. This will involve a significant amount of well-designed experimentation and is more easily done when one understands the physical and chemical processes occurring within the sensor system, especially rate-limiting diffusions and immunological binding considerations. This understanding can often be gained by examining similar sensors which have been described in literature or by obtaining comprehensive reports regarding the testing that a contractor may have already performed on the sensor under study. This information will allow for some conceptual modeling to occur which can effectively direct and delimit

the empirical investigations that yet may be required. Only those parameter ranges that are expected to occur in actual sensor use, or that can be maintained through external means during actual use, need to be investigated.

The dependence of the sensor baseline reading on the different reaction parameters is measured by keeping the analyte concentration constant and selectively varying each of these other parameters. The effect of different reaction conditions on the magnitude and speed of sensor response to changes in analyte concentration is determined by performing a series of analyses such as those described in Section 2 under several different reaction conditions. A "simplex"<sup>9</sup> or other type of systematic experimental design should be used in order to investigate plausible ranges of reaction parameters. The simplex design allows the simultaneous optimization of several parameters by making only a reasonable number of measurements.

## 6. EXCITATION AND REFERENCE ELEMENTS

The utility of precalibrations of the sensing system will be dependent on the inherent stability of both the sensor itself and any excitation sources or reference elements in the system. These latter components include light sources, reference electrodes, reference streams in a flow system, and reference crystals in a piezoelectric sensor system. It is desirable for a reference element in a sensor system to have very high absolute stability, or for it to have properties which are very predictable or which closely match those of the sensor itself (except for sensitivity to the analyte of interest). Only in such cases will the difference between sensor and reference element outputs have quantitative significance. In any case, it is advantageous to determine the reference element's properties independently from those of the sensor. This will usually explain the exact source of temperature dependence, pH sensitivity, reaction to interferences, etc., that may have been observed for the system as a whole. For a full evaluation of the reference element, tests such as those described in Sections 2 through 5 would need to be performed in which the reference element is monitored against an independent method or reference element (standard) of known absolute behavior. Table 5 lists some specific tests that can be performed on different types of excitation or reference elements. It is usually much easier to improve reference elements (or to replace them with elements of higher quality) than it is to modify the sensing elements, since the reference elements are generally of more simple construction than the latter and are often available from commercial sources.

Table 5. Tests Appropriate for Reference Elements

---

1. Reference Electrodes (tested against another reference electrode connected to the sample solution by a very stable and insensitive liquid junction)

thermal sensitivity  
random noise and drift  
sensitivity toward changes in ionic strength  
sensitivity toward analytes and interferents  
effectiveness of rinsing and washing procedures

2. Reference Piezoelectric Crystals (tested against a high-quality frequency counter)

thermal sensitivity  
random noise and drift  
sensitivity toward analytes and interferents (if the crystal is exposed to the sample during normal operation)  
time to regain baseline after sample exposure, if applicable

3. Light Sources, Reference Beams (tested against a high-quality photodetector)

thermal sensitivity  
random noise and drift  
aging effects

4. Reference Channels in Flow-through Redox Systems (tested against an independent analytical method or formulation)

sensitivity toward analytes and interferents  
effectiveness of rinsing and washing procedures

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Many of the processes that the chemical and biological sensors will be monitoring or using as a detection mechanism have complex, multi-phasic, and often slow kinetic properties (e.g.,  $\tau > 1$  minute, where  $\tau$  is the time required for the signal to have effected 63% of the total change that it makes upon a step change in concentration or other input parameter). It is generally desirable to read the sensor output at the first point in the cycle of sample analysis where the sensor output can be shown to be a reproducible and sensitive indication of analyte concentration because this will allow for maximum sample throughput. This point may or may not be a point of chemical or physical equilibrium or steady state. Figure 3 presents data collected from a pH electrode in two types of analyses, illustrating this concept. In the first, the electrode was allowed a long period of time to equilibrate with a sample; in the second, samples were rapidly passed by the electrode in a carrier stream to produce peaks in the electrode potential, the size of which are proportional to the pH and buffer capacity of the samples.

Table 6 presents recommended sample handling procedures to use with sensors having different kinetic properties. In this context, the kinetic behavior of the sensor is defined as that of the selective coating, liquid junctions, or other surface phenomena directly associated with the sensor. Table 6 includes cases where independent reactions of varying kinetic character occur prior to the detection step at the sensor, as well as the case where there is no pre-reaction. The recommendations are based on the fact that reactions monitored before reaching completion produce a smaller signal, reducing the signal/noise ratio; thus, the ultimate analyte sensitivity (lower detection limit) is sometimes not as good as it would be using stopped-flow or other types of equilibrium measurement.

In order to use nonequilibrium measurements, one must be able to precisely reproduce the conditions on which the kinetics of the chemical or biological reaction depend. In "open beaker" or stopped-flow techniques where chemical reactions are allowed to proceed to near equilibrium or steady-state response; reaction kinetics are of less importance since the sensor output will usually change rapidly at first, then more slowly, often exponentially approaching a steady-state value. Measurements of the sensor output are made on the response "plateau" and are thus not extremely time sensitive.

For nonequilibrium measurement, a reproducible method of presenting the sample to the sensor must be devised in order that this step may be used as a temporal reference point. Flow injection analysis<sup>10</sup> or other analytical methods incorporating automatic sample injection can be used (Figure 4). After developing an adequate sample presentation method, the other parameters of the sample-handling procedure can be optimized. Several analyses

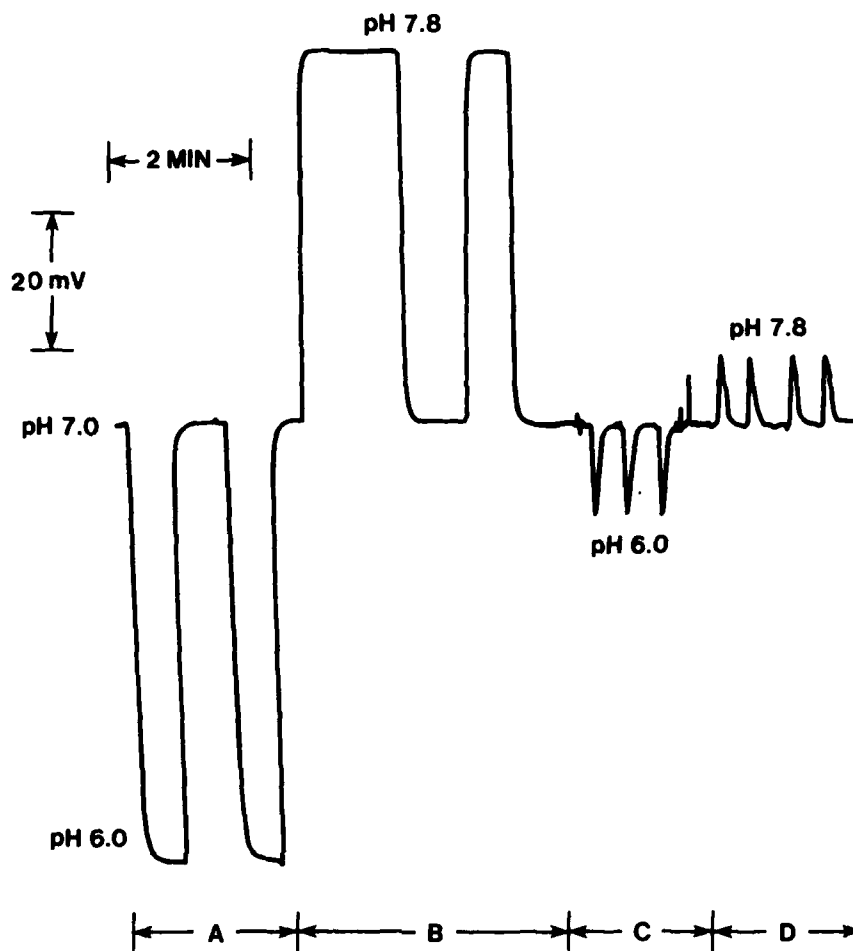


Figure 3. Response of a pH Electrode. A. Switching between buffer solutions of pH 7 (center baseline) and pH 6; B. Switching between pH 7 and pH 7.8 buffers; C. 0.02-ml samples of pH 6 buffer injected into a flowing carrier stream of pH 7 buffer; D. 0.02-ml samples of pH 7.8 buffer injected into flowing carrier stream.



Table 6. Sample Handling Procedures to Use in the Coupling of a Chemical Reaction to a Subsequent and Separate Detection Step

| Type of reaction   | Very slow sensor<br>$\tau > 1 \text{ min}$ | Slow sensor<br>$1 \text{ min} > \tau > 0.5 \text{ sec}$ | Fast sensor<br>$\tau < 0.5 \text{ sec}$                               |
|--|--|---|---|
| very slow reaction<br>$\tau > 1 \text{ min}$                 | stopped flow at sensor                     | stopped flow at sensor                                  | incubation, followed by FIA* across sensor; or stopped flow at sensor |
| slow reaction<br>$1 \text{ sec} > \tau > 0.5 \text{ sec}$    | stopped flow at sensor                     | stopped flow at sensor                                  | reaction coil or incubation, followed by FIA across sensor            |
| fast reaction or no pre-reaction<br>$\tau < 0.5 \text{ sec}$ | stopped flow at sensor                     | FIA or stopped flow at sensor                           | FIA (will give near steady-state, diluted at interfaces)              |

\*FIA: Injection of sample into a flowing carrier stream

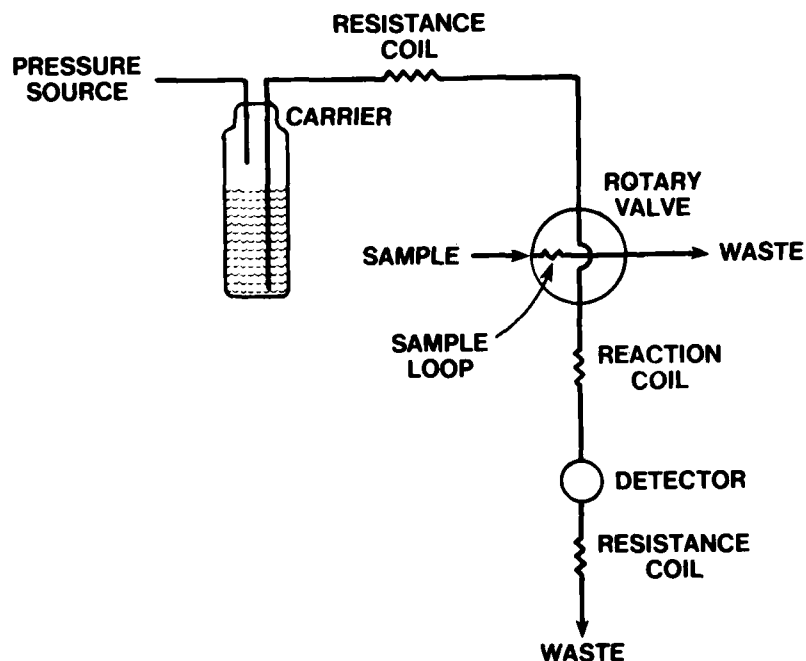


Figure 4. Typical Flow Injection Analysis System. Sample is loaded into sample loop with rotary valve at position shown. Rotating valve by 90° injects controlled volume of sample into flow stream.

must be performed with samples of several different analyte concentrations in which sensor response with respect to time is monitored. Statistical analysis of these kinetic runs will reveal how well the sensor output at any particular time after sample presentation can predict analyte concentration. In flow-injection analysis, flow rates, mixing conditions, and incubation times will need to be optimized so that peak height, rate of change in sensor output at a particular time, or another monitored parameter<sup>11</sup> is most reflective of analyte concentration. A trade-off between maximum sensor performance, minimum sample processing time, and optimal reagent consumption will often have to be made to determine the point at which the sensor output should be read.

## 8. SENSOR STORAGE AND CONDITIONING

This section contains information that more appropriately relates to the design of measurement procedures to follow in using a sensor, rather than to the testing of an established procedure. However, the information is included to emphasize the importance of storage, preconditioning, and rinsing conditions in the operation of many surface-active sensors. Empirical testing related to these concepts should be aimed at determining the adequacy of an established method (such as that suggested by the developer of the sensor) and at investigating alternative methods in order to find the most effective and practical means of treating the sensors before and in between analyses.

### 8.1 Storage.

The preferred method of storage for a particular micro-sensor will depend on the active surface of that sensor. If the active surface must be hydrated to be functional, then the sensor will either have to be stored in solution or in a saturated water vapor environment, or the sensor will have to be preconditioned before actual use by soaking in some type of buffer solution. Usually a phosphate or acetate buffer is used for storage with sodium azide (0.2%) or another preservative added to prevent microbial growth. If the active surface of the sensor does not need to be hydrated, or if the preconditioning time required to obtain functionality is short, the sensors would probably be stored in a dry condition to minimize the leaching away or the hydrolysis of critical surface entities. Complications involved in determining an effective storage method include the fact that some proteins denature when completely dried or when dried in an improper manner. Surface coatings can shrink and break loose from sensor surfaces when dried. Water can seep in between the surface coating and the sensor surface (affecting the transduction ability of the sensor) when the sensor is stored in solution. In addition, sensors depending on an ion-exchange, gas permeability, or other similar phenomenon should be stored either dry or in a solution containing a moderate concentration of the critical substance (ion, gas, etc.).

## 8.2      Preconditioning.

The length of time needed for preconditioning is determined experimentally by observing when the sensor response begins to be acceptable and when the rate of sensor drift falls to its normal-operation value. If a significant amount of time is required for preconditioning (more than a few minutes), and the sensor lifetime after conditioning is short (less than a day), the sensor would not be convenient for use on the battlefield.

## 8.3      Special Considerations for Multiuse Sensors.

When a sensor is to be used for several assays over an extended period of time, experiments should be conducted to determine sensor lifetime. Sensor lifetime refers to the amount of elapsed time or number of assays that can be performed before the sensor response to changes in analyte concentration decays to an unacceptable level. Detection of an analyte is considered acceptable only when the sensor response to a sample is greater than three times the variance of a blank determination for a sample containing analyte at or below the analyte's established minimum detection criterion.

An important procedural element in using sensors for multiple assays is the washing or rinsing cycle between assays. This process serves three functions:

- a. Reestablishes baseline conditions to allow reproducible analyses based on exact timing of the initiation and measurement of a fast reaction.
- b. Periodically recalibrates the sensor with a solution of known concentration.
- c. Rinses sensor surfaces of interfering materials.

If the first function is important in the sensor system, the washing/rinsing step must be performed between each assay. If only functions b. and c. are important, and the sensor reaction mechanisms are rapid and easily reversible, the rinsing step can be performed less frequently. In this case, flushing with a solution of defined composition close to that of a typical sample (such as pure sample carrier, which might be a pH buffer solution) is usually adequate. Flushing must be done at intervals determined by the typical rate of drift in the sensor output and by the anticipated rate of coating of the sensor by interfering substances. The need for rinsing can also be based on the sensor's response to a recalibration attempt.

If the sensor processes and reactions are slow or not easily reversible, the rinse cycle must be prolonged or a wash solution of more harsh composition must be used which is capable of rapid surface regeneration. Selective surfaces and reference

electrodes may need to be cleaned of adsorbed materials, and some surface reactions (such as oxidations or reductions) may need to be reversed. In addition, substances that may have diffused into a surface layer may need to be leached back out again to reestablish a previous baseline condition. The results of one assay will tend to depend on the results of the previous assay unless adequate washing and rinsing are performed between each assay. The wash solution may contain ionic detergents such as sodium laurel sulfate, nonionic detergents such as tyloxapol, other surfactants, solvents such as alcohols, chelating agents such as disodium EDTA, and acidic or basic pH buffers. It should be noted that the wash solution should not irreversibly affect the sensor, and that the sensor's reaction time is usually greater, the greater the difference in analyte concentration between the sample and the wash/buffer solution. Harsh wash solutions should be rinsed out with a solution of defined composition close to that of a typical sample before the next sample is presented to the sensor.

Operationally, the time required to wash, rinse, and possibly recalibrate the sensor between assays is often referred to as reset time (though reset time can also be determined by the sample acquisition time if this is longer than the combined analysis and rinsing time or if sampling cannot be done concurrently with analysis and rinsing). It is desirable to find the most effective washing/rinsing procedure, with respect to time and function, as this step is obviously a major determinant of the maximum possible sample throughput in the sensor system.

## 9. SIGNAL PROCESSING

Usually some type of amplification or other analog signal processing will be involved in the sensor system. Typically, the analog processing elements are much more stable and dependable than the other portions of the sensor system; but these components cannot be entirely ruled out as significant contributing factors to the total drift or random noise in the system, and tests of their stability should be made early in the sequence of system testing. This can be done by substituting a stable element such as a wire-wound resistor, a battery, etc., for the sensor itself, and noting the output signal from the amplifier as a function of time and in response to situations that could be expected to arise in normal use, such as power surges, electrostatic coupling, vibration, and thermal variations.

Microprocessor-controlled sensor systems will incorporate digital control of mechanical elements and probably digital processing of the sensor signal. The software should be tested to determine if any malfunctions or idiosyncrasies exist.

## 10. PHYSICAL DESIGN

The sensor system, including associated sample and reagent-handling hardware, electrical components, and the sensing

element itself, should possess (or be capable of being upgraded to possess) certain physical characteristics important for the system's success in advanced development testing and Army field use. Some of the factors that need to be investigated, at least qualitatively, are sensor lifetime, sensitivity to vibration or physical shock, temperature and light sensitivity, and susceptibility to electrostatic coupling. Some of this testing may have been completed in the course of the other tests previously described (see Sections 3 and 5). The results of these investigations and the effectiveness of any compensatory controls developed (such as electromagnetic shielding, temperature control or compensation, shielding from light, etc.) should be recorded.

Other obvious considerations, such as size, weight, power, reagent, and sample requirements also need to be evaluated to determine if they are compatible with field use or if they can be easily modified to meet field requirements. Standards (or goals) for these parameters were published<sup>12</sup> for different field applications. It should be noted that these standards are for fielded devices and are not necessarily expectations for advanced development prototypes.

#### 11. ADAPTABILITY

Although not a formal evaluation of the sensor behavior itself, a significant aspect of the sensor system is its capability of being easily adapted to sense substances closely related to the analyte for which it is being evaluated. Although evaluating the adaptability of a sensor system to new, perhaps presently unknown substances may be somewhat subjective, there are some objective items to consider in making such an evaluation.

Adaptability can be evaluated on two fronts. The same sensing mechanism can be used for the detection of different substances through the substitution of different sensor coatings, different antibodies, etc., on the original sensing element; or different sensing mechanisms can be used by adapting an entire sensor system (including peripheral components) to the possible substitution or addition of similar or dissimilar sensing elements.

In regard to the first of these considerations, it is important to determine the theoretical and practical limitations of the sensing mechanism. Table 7 presents some applicable considerations for sensors based on various standard sensing mechanisms. In this evaluation, distinction should be made between specific sensing capabilities that have actually been demonstrated and those that have just been postulated.

Table 7. Constraints on the Application of Various Sensing Mechanisms

| Sensing Mechanism                                | Constraints on Method Application   |
|--|---|
| 1. Competitive binding assay (general)           | <ul style="list-style-type: none"> <li>-Need for antibodies with adequate binding constants and kinetic properties</li> <li>-Must be able to immobilize antibody or antigen on solid surface without affecting activity</li> </ul>  |
| 2. Fluorescent-labeled immunoassay               | <ul style="list-style-type: none"> <li>-Must be able to label antibody or antigen without affecting immunological activity; necessary functional group must exist at a noncritical portion of the antibody or antigen</li> <li>-Quantum yield of fluorophore must be sufficient after binding to antigen or antibody</li> </ul> |
| 3. Enzyme-coupled indirect immunoassay (general) | <ul style="list-style-type: none"> <li>-Enzyme must retain sufficient activity when bound to antigen or antibody</li> </ul>   |
| a. Potentiometric assay                          | <ul style="list-style-type: none"> <li>-Subject to differences in pH and temperature</li> <li>-Sensitive to natural presence of product or other interferents</li> </ul>  |
| b. Amperometric assay                            | <ul style="list-style-type: none"> <li>-Subject to differences in pH and temperature</li> <li>-Sensitive to natural presence of product or other interferents</li> </ul>  |
| c. Spectrophotometric assay                      | <ul style="list-style-type: none"> <li>-Subject to interference from substances naturally present in sample or carrier absorbing at the detected wavelength</li> </ul>  |
| 4. Direct potentiometry using a redox polymer    | <ul style="list-style-type: none"> <li>-Analyte or secondary messenger must be capable of reducing or oxidizing the redox polymer</li> <li>-Redox polymer will be subject to oxidation or reduction from other species</li> <li>-Some reactions will be irreversible</li> </ul>   |
| 5. Direct amperometry (general)                  | <ul style="list-style-type: none"> <li>-Operating potential range is limited by solvent electrolysis</li> <li>-Subject to interference by other oxidizable or reducible substances</li> </ul>   |
| a. redox at a set potential                      | <ul style="list-style-type: none"> <li>-Electrode surface needs renewal</li> <li>-Product must be removed</li> <li>-Sensitive to irregular mixing</li> </ul>  |

Table 7. (Continued)

| Sensing Mechanism                     | Constraints on Method Application   |
|---------------------------------------|---|
| b. differential pulse<br>voltametry   | -Subject to interference only by those<br>substances oxidized in the range over which<br>the potential is stepped                         |
| 6. Gravimetric piezoelectric<br>assay | -Lower molar sensitivity with analytes of<br>lower molecular weight<br>-Need for surfaces at least partially<br>selective for the analyte |

In order for the sensor system as a whole to exhibit adaptability, it should be modular in design so that the selective component of the sensor system can be removed and replaced by a similar (or perhaps dissimilar) component which is selective for the same or a different analyte. The range and gain of the electronic amplifier or other signal processing components should be adjustable to allow for changes in baseline signals and sensor sensitivity occurring with the replacement of the sensing element. Reagent storage and delivery capabilities should be adequate to accommodate different sensing applications and perhaps even different sensing mechanisms. Reagent flow rates and reaction incubation times should be capable of being modified to optimize the measurement of different analytes. Obviously there will be a limit to this adaptability, due to the increasing complexity inherent in the incorporation of an increasing number of options.

If the sensor system does not seem to be adaptable in its present configuration, an evaluation should be made of the ease with which the system could be modified to make it adaptable. Also of interest is the ease with which the system could be expanded to incorporate several different sensors, without adversely affecting the reliability and functionality of the system. With regard to this latter consideration, it is important to determine sample and other reagent volumes needed and the time that would be required to run the multiple analyses in the upgraded system; these parameters would be partially determined by whether the analyses would be run in series, in parallel, or a combination of these two.

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